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BRIEF COMMUNICATION

TIMING OF SEED DISPERSAL GENERATES A BIMODAL SEED BANK DEPTH DISTRIBUTION[1]

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The density of soil seed banks is normally highest at the soil surface and declines monotonically with depth. Sometimes, for a variety of reasons, peak density occurs below the surface but, except in severely disturbed soils, it is generally true that deeper seeds are older. In seasonally dry habitats that develop deep soil cracks during the dry season, it is possible that some seeds fall down cracks and rapidly become deeply buried. We investigated this possibility for three dominant clonal perennials (*Scirpus maritimus*, *S. litoralis*, and *Juncus subulatus*) in the Doñana salt marsh, a nontidal marsh with a Mediterranean climate located in southwest Spain. Two species, which shed most of their seed during the dry season and have seeds with low buoyancy, had bimodal viable seed depth distributions, with peak densities at the surface and at 16–20 cm. A third species, which shed most seeds after soil cracks had closed and had seeds with high buoyancy, had viable seeds only in surface soil. Bimodal seed bank depth distributions may be relatively common in seasonally dry habitats with fine-textured soils, but their ecological significance has not been investigated.

Key words: Doñana; helophytes; *Juncus*, Mediterranean wetland; *Scirpus*; seed buoyancy; seed rain; soil cracks.

Depth distributions of viable seeds are not of merely academic interest. The ability to predict the distribution and emergence of viable seeds, following different forms of soil disturbance, can be vital for both weed control and vegetation restoration. In the overwhelming majority of published accounts, soil seed bank density declines monotonically with soil depth. This pattern is assumed to reflect regular seed input at the surface and a more-or-less gradual decline in viability as seeds both age and move vertically down the soil profile. Because older seeds have more time to become deeply buried, depth distribution is often a reasonably good indicator of seed longevity (Thompson et al., 1997; Bekker et al., 1998). Seed bank distributions with a peak density below the soil surface are usually assumed to have a historical explanation, reflecting a seed input that was greater at some time in the past and has subsequently declined. Examples include the presence of deeply buried seeds of arable weeds under formerly cultivated pasture (e.g., Chippindale and Milton, 1934) and deeply-buried seeds of pioneer species in the later stages of succession (e.g., Bekker et al., 1999). The assumption that deeper seeds are older may also be violated, especially in cultivated soils, and cultivation by different implements may lead to quite complicated depth profiles, with mixtures of seed ages at all depths (Grundy et al., 1999).

Climates with alternating dry/wet seasons frequently develop vertic soils, that is, fine-textured soils that experience extreme changes in key soil properties throughout the year and tend during the dry season to develop cracks of varying width and depth, which become closed during the wet season (Eswaran et al., 1999). Frequently, the opened cracks tend to be filled with materials from the surface that are dislodged by animals, wind, or water, and these can become trapped when the cracks close. These ''internal movements'' in the soil redistribute materials (churning, or horizon disruption, Buol et al., 1989) in the horizons affected by cracking, which may affect the distribution of plant propagules. Vertic soils are distributed worldwide and predominantly under a natural vegetation of salt marshes, grasses, savannas, open forest, or desert shrubs (FAO-ISRIC-ISSS, 1998; Soil Survey Staff, 1999).

Little is known about how much (1) cracks influence the vertical distribution in the soil of seeds from different species, (2) timing and velocity of seed release could affect the final distribution of the seed bank of different species, and (3) trapped seeds can preserve their viability after favorable conditions are reestablished.

Here we determine the impact of seasonal soil cracking on the distribution of the soil seed bank in a Mediterranean wetland. We hypothesize that, in contrast to the overwhelming majority of reports in the literature (Thompson et al., 1997), the viable seed bank of some species may have a bimodal distribution (U-shaped). We also discuss the roles of soil cracks and primary dispersal syndrome (i.e., time of seed release and seed buoyancy) in generating this distribution.

Study site—During the summer, soils of wetlands under Mediterranean climates (frequently calcareous, saline, and clayey) develop cracks, which then close in autumn during the wet season. In Doñana salt marshes, a nontidal marsh with a Mediterranean climate located in southwest Spain (37° N, 6° W), areas colonized by perennial plants have abundant cracks in the soil (especially in the range 0–20 cm depth, Clemente et al., 1998) during the dry season, when many species, including some perennial emergent macrophytes, shed their seeds.

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TABLE 1. Study of the salt marsh dominants *Scirpus maritimus*, *S. litoralis*, and *Juncus subulatus*. Biomass and seed production during 2001–2002 hydrological cycle, seed characteristics (mass; $N = 100$), and dispersal (seed buoyancy; $N = 4$ and seed release); t_{50} = number of days until 50% of seeds sink, t_{100} = number of days until 100% of seeds sink. The data are means ± SE. See Fig. 1 for information about seed release.

Species	Biomass ($\cdot 10^3$ kg $\cdot$ m^{-2})	Seed production ($\cdot 10^4$ seed $\cdot$ m^{-2} year^{-1})	Weight ($\cdot 10^{-4}$g; $N = 100$)	Bouyancy (days)	Seed release
S. litoralis	2.738 ± 0.33	8.64 ± 1.66	7.38 ± 0.02	Low t_{100} = 2.25 ± 0.47	fast
S. maritimus	2.16 ± 0.43	1.17 ± 2.71	25.08 ± 0.66	High t_{50} = 29.5 ± 3.59; t_{100} = 61 ± 3.53	slow
J. subulatus	1.85 ± 0.28	188.03 ± 28.29	0.26*	Low t_{100} = 1.75 ± 0.47	medium

Study species—The major components of the vegetation in Doñana temporary marshes are the rhizomatous and perennial emergent macrophytes *Juncus subulatus* Forsskal, *Scirpus maritimus* L., and *S. litoralis* Scharader. *Juncus subulatus* inhabits shallow brackish waters in coastal areas of the Mediterranean and Irano-Turacic regions (Valdes et al., 1987). *Scirpus litoralis* Scharader and *S. maritimus* L. (Cyperaceae) are more widely distributed in shallow, brackish water bodies of temperate regions (Valdes et al., 1987). In all three species, belowground parts sprout once the wet season begins in autumn. The shoot grows up through the water column, emerging in autumn (*J. subulatus*) or winter (*Scirpus* spp.), fruits through the spring, and dies in the dry season (summer). Previous studies (García et al., 1993; Espinar et al., 2002) have shown that these geophytes become dominant in different zones of the microtopographic gradient, with *J. subulatus* occupying the highest parts, which are flooded to a lesser depth and for a shorter time, while *S. litoralis* uses the deepest areas, flooded longest. *Scirpus maritimus* occupies intermediate areas.

MATERIALS AND METHODS

Reproductive data—In autumn 2000, a single population of each species was chosen for the study, and within each population two locations (study plots separated by at least 1.5 km) were selected for construction of a fence with an exclosure (625 m^2) to protect them from cattle (principally to avoid herbivory of fertile shoots and to avoid soil mixing by trampling). In late spring of 2002, three cores (of soil and vegetation) were collected in each population (0.16 m diameter and 0.2 m depth; volume = 0.004 m^3) and were separated into above- and belowground biomass and flowering spikes. To obtain a description of the population, the biomass was dried to a constant mass and weighed. The annual production of seed per species was measured by direct count of seeds in the flowering shoots collected. One hundred seeds of each species were selected at random and weighed to obtain a mean seed mass. Another 100 seeds were utilized in a buoyancy test. The seeds were separated into groups of 25 seeds and placed in 100-mL plastic pots filled with tap water. Every day the pots were shaken and floating seeds counted (Hroudova et al., 1997).To estimate the rate of seed loss from fertile shoots of all three species, shoot seed content was sampled in June, August, and early November. From each study population, the inflorescences of 10 shoots were collected at random, with each shoot separated from each other by at least 1 m. In the laboratory, the number of seeds per shoot was counted (in three spikes per inflorescence for *Scirpus* species and three capsules per inflorescence for *J. subulatus*).

Seed bank estimation—In October 2002, at the beginning of the wet season, three soil cores were collected in two study plots for each species (6 cores per species; 0.08 m diameter and 0.16 m depth). Each core was carefully separated (to avoid contamination between different depths) into four depths (0–0.04, 0.04–0.8, 0.08–0.12, 0.12–0.16 m) and placed in separate bags. Each sample was air dried and carefully homogenized by hand in the laboratory. To obtain a complete inventory of seeds, we obtained three sets of data for each sample: ''by germination,'' followed by sieving for ''direct counts,'' and then following cold treatment a second germination period for ''recovery.'' A 0.05-kg subsample of each homogenized sample was placed in a 14-cm petri dish with demineralized water until soil saturation. The petri dishes were closed to avoid loss of humidity and stored in the dark at 4°C for 30 days to stimulate germination (Clevering, 1995; Espinar et al., 2004). After this period, demineralized water was added to each petri dish until the soil was covered. Dishes were then placed in a germination chamber with a 12 h–12 h light–dark photoperiod, photon flux of 500 μmol photons/m^2 and temperature cycle of 25°/15°C. Every 3 days, the soil was disturbed and the number of seedlings in each dish was counted (and removed) to obtain, after 60 days, the soil seed bank density ''by germination.'' After germination, the same soil sample was sieved and the number of intact seeds was counted, to obtain the soil seed bank density ''by direct counts.'' The soil samples were not dried before sieving. To detect the viability of seeds revealed by direct counting, they were placed in a freshwater medium for a month at 4°C in darkness, then placed in a petri dish containing a Whatman No. 1 filter paper (Maidstone, UK) and 20 mL demineralizd water. Germination was tested under the conditions described; every 3 days for 30 days, the number of seeds germinated on each dish was counted to obtain the total percentage germination ''by recovery.''

Data analysis—Variation of all study variables with depth was analyzed using a nonparametric test (Kruskal-Wallis nonparametric ANOVA). The analyzed dependent variables did not meet the assumption of normality and homogeneity of variance necessary for a parametric ANOVA. For each species, the relationships between the different seed variables and depth were first explored using a local regression procedure (distance weighted least squares regression lines (DWLS) are shown; McLain, 1974). When we found significant differences in the Kruskal-Wallis test, we were interested in testing if a U-shaped model of seed bank depth distribution significantly improves on a monotonic decreasing one. That is, when a positive one-tailed test for the quadratic polynomial coefficient (a_2) was positive, a test of H_0: $a_2 = 0$ vs H_1: $a_2 > 0$ (where H_0 is the null hypothesis and H_1 is the alternate hypothesis) was performed. To test the U-shaped model, we using a generalized linear model (GLM), assuming a Poisson distribution (given the nature of the analyzed dependent variable—counts) and a log link function (Dobson, 2002) using depth as the independent variable. The increased type 1 error rate by repeated testing was controlled at an overall 0.05 level by using the Holm sequential Bonferroni procedure (Holm, 1979; García, 2004).

RESULTS

Species descriptions—The three species showed different values of the measured reproductive parameters, seed size, seed floating ability, and seed release (Fig. 1, Table 1).

Seed bank distribution—The result of the seed bank measurements are shown in Table 2. The seed bank estimate ''by germination'' represents the entire viable seed bank, except for *S. litoralis* when the recovery test showed a few viable seeds among those estimated by direct count in the 0–4 and 8–12 cm depths (i.e., here the viable seed bank = estimate by ger-

TABLE 2. Soil seed banks (no. seeds/m^2) at different depths of *Scirpus maritimus*, *S. litoralis*, and *Juncus subulatus*. Viable seed density (estimated by germination + recovery test); no viable seed bank (estimated by direct counts − recovery test) and the result of the Recovery germination tests performed on intact seeds gathered by directs counts (Recovery test) are shown. The data show mean ± SE. The result of Kruskal-Wallis ANOVA test is also showed (significance level $P < 0.05$).

Species	Variables	Soil depth (cm)				Kruskal-Wallis ANOVA	
		0–4	4–8	8–12	12–16	*H*	*P*
S. litoralis	Viable seeds	3486 ± 665	1509 ± 679	302 ± 191	1252 ± 433	11.84	0.0079
	Nonviable seeds	13 286 ± 6602	9122 ± 3913	26 105 ± 9615	46 385 ± 1931	3.90	ns
	Recovery test	84 ± 84	0	158 ± 158	0	—	—
S. maritimus	Viable seeds	2256 ± 910	0	0	0	—	—
	Nonviable seeds	5218 ± 2308	1700 ± 1255	1243 ± 762	3462 ± 1824	3.59	ns
	Recovery test	0	0	0	0		
J. subulatus	Viable seeds	2897 ± 796	360 ± 360	455 ± 279	1033 ± 1033	7.85	0.048
	Nonviable seeds	6584 ± 2455	4390 ± 2272	924 ± 681	4364 ± 1907	4.63	ns
	Recovery test	0	0	0	0	—	—

mination + seed recovery of direct counts). No seeds of *S. maritimus* and *J. subulatus* measured by ''direct count'' germinated in the recovery test. In the case of *S. maritimus*, although we found seed at all depths, viable seeds appeared only in the top soil (0–4 cm). Overall, nonparametric Kruskal-Wallis ANOVA indicated that, both for *S. litoralis* ($H = 11.8$, $P < 0.008$) and *J. subulatus* ($H = 7.9$, $P < 0.048$), there were differences in viable seed density between the sampled depths (Table 2). For both species, local regression curves suggested (Fig. 2, DWLS fits) that the occurrence of viable seeds followed a U-shaped distribution, with peaks at the surface (0–4 cm) and deepest (12–16 cm) layers, instead of the monotonic decreasing trend, which is usually observed in uncracked, undisturbed soils. The result of the test of the U-shaped model by GLM (test for the quadratic polynomial coefficient being positive, i.e., $H_0 : a_2 = 0$ vs. $H_1 : a_2 > 0$) is shown in Table 3. In both *S. litoralis* and *J. subulatus*, the occurrence of viable seeds showed a significant U-shaped distribution, with peaks in the surface (0–4 cm) and deepest (12–16 cm) layers.

DISCUSSION

Primary dispersal syndrome and viable seed depth distribution—Although we found seeds of each species in all study depths, in contrast to most reports in the literature (Thompson et al., 1997), the viable seed bank of two study species (*S. litoralis* and *J. subulatus*) shows a bimodal distribution, with seeds found at all depths, but highest densities in topsoil and in the deepest samples. This pattern could be associated with early seed release in *S. litoralis* and *J. subulatus*. *Scirpus maritimus*, which inhabits the same area, but has a more delayed seed release, has viable seeds only at the soil surface. We suggest that the entry of seeds into deep soil layers via cracks in dry soil during the period of summer seed rain for *S. litoralis* and *J. subulatus* (but not for *S. maritimus*) could be the fundamental cause of this depth distribution. In addition to this effect of cracks, the buoyancy capacity of seed plays an additive role in the process, because the high floating capacity of *S. maritimus* seeds means that the seeds that could fall into the cracks (about 40% of seed production) can easily float out again when flooding begins at the start of the wet season. Seeds of *S. litoralis* are mostly released, while cracks are still open, sink fast in water, and are easily trapped in the soil (see Fig. 1).Thus the U-shaped depth distribution in *S. litoralis* and *J. subulatus* is linked to the fact that the average depth of soil cracks in the study area is around 20 cm (Clemente et al., 1999), and the majority of seeds that fall into cracks end up at or near the bottom of the cracks. During the dry season, these cracks are partially filled with superficial soil materials and a noticeable number of seeds, which become trapped in the soil when the cracks suddenly close at the beginning of the wet season. Although viable seeds of *S. maritimus* were found only in the surface soil, we found large numbers of nonviable seeds of all three species at all depths. Seeds of *S. maritimus* may be short-lived, or they may suffer a rapid loss of viability when buried, but we have no data on seed longevity or the effects of burial on seed viability. After several or many cycles of swelling—shrinking, the soil becomes ''inverted,'' and subsoil materials may eventually come to the surface (Buol et al., 1989) and interact with light and less saline water. However, it is difficult to evaluate the percentage of deeply buried seeds that reach the soil surface via this route, and the time period involved in the process.

Ecological implication of burial seeds—The ecological implications of the different capacity of seeds for burial remain unexplored, but are probably different in each study species.

TABLE 3. Result of generalized linear model (GLM) analysis of the number of viable seeds (estimated by direct germination + recovery test) in *Scirpus litoralis* and *Juncus subulatus* in the soil seed bank. Data have been modeled using a GLM, assuming a Poisson distribution, log link function, and both linear and quadratic effects of soil depth (D, D^2). All coefficients were statistically different from zero, after applying a sequential Bonferroni correction. Boldface values are significant.

Species	Variable	Effects	Factors			
			Coefficient	SE	Wald's χ^2	*P*
S. litoralis	Viable seeds	D	−0.691	0.269	6.588	**0.005**
		D^2	0.029	0.013	4.575	**0.016**
J. subulatus	Viable seeds	D	−1.00	0.483	4.28	**0.019**
		D^2	0.004	0.024	3.48	**0.031**

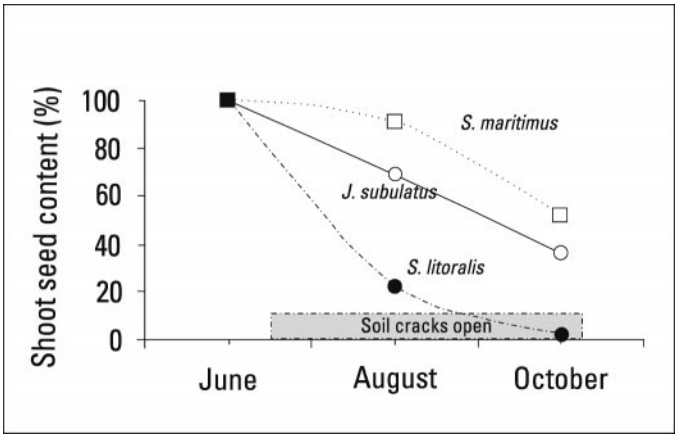

Fig. 1. Seed release measured as the percentage of original shoot seed content (seed per spike or capsule) over time for the three study species. The broken-line rectangle in the *x*-axis indicates the dry period with open soil cracks.

Although seeds of both *Scirpus* species are usually consumed by birds (Espinar et al., 2004), *S. maritimus* seeds are bigger than *S. litoralis*, and *S. maritmus* can float at the water surface for several weeks. Both facts suggest that *S. maritimus* seed are more susceptible to predation than *S. litoralis*. Furthermore, the timing of massive seed release of *S. maritimus* coincides with the presence of large migratory birds. The ratios of seed production by *S. maritimus* (Sm), *S. litoralis* (Sl), and *J. subulatus* (Js) are nearly 1 : 8 : 188 (Sm : Sl : Js); yet the ratios of their viable seeds in the upper 4 cm (with proven ability to germinate and establish) are nearly 1 : 1 : 1. Considering only seed that could germinate and establish and the importance of the local seed bank, the processes described in this paper might seem to favor the dispersal syndrome of *S. maritimus* (i.e., with a low production of seed yet a local seed bank with the same density of viable seeds) rather than the other two species. As mentioned, both the eventual fate and ecological significance of deeply buried seeds in *S. litoralis* and *J. subulatus* remain unknown. Clearly, we need to discover the capacity of buried seeds to return to the surface and the possible loss of viability in buried seed. An important reserve of viable seed exists only in *S. litoralis* and *J. subulatus*, and buried seeds of these species are protected from predation. On the other hand, seeds that fall into cracks are subject to different conditions of humidity and salinity (increased exposure to moisture and different salinity levels) than seeds that remain at or near the surface. The duration of exposure to salinity and moisture, as tested in laboratory conditions, has a significant effect on seed germination pattern in all three study species (Espinar et al., 2005), but the effects under field conditions remain unknown. There are several reports of a persistent seed bank in *S. maritimus* (Thompson et al., 1997). There is no published information on *S. litoralis* or *J. subulatus*, but all species of *Juncus* appear to have persistent seed banks.

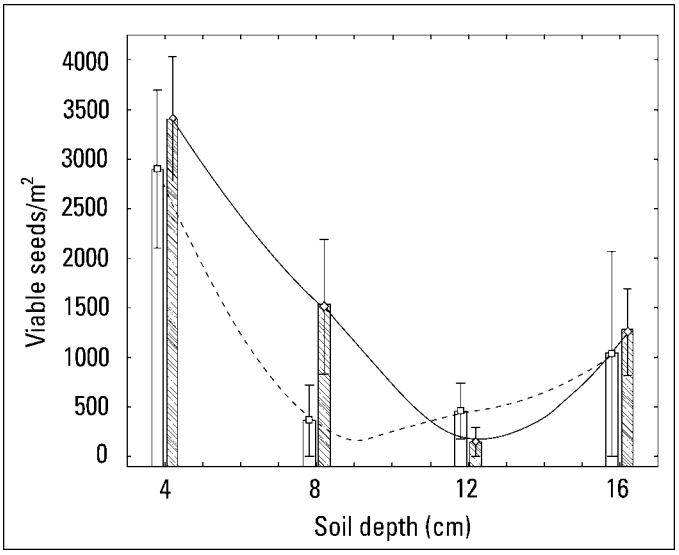

Fig. 2. Viable seed (estimated by germination + recovery test) at different soil depth. Bars indicate mean values ($N = 6$) and standard error (white bars for *Juncus subulatus* and filled for *Scirpus litoralis*). The lines (broken for *J. subulatus* and continue for *S. litoralis*) indicate the result of exploratory analysis using a local regression procedure, DWLS (distance weighted least squares regression; McLain, 1974).

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